Insights into the Mechanisms of Ifosfamide Encephalopathy: Drug Metabolites Have Agonistic Effects on \( \alpha \)-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA)/Kainate Receptors and Induce Cellular Acidification in Mouse Cortical Neurons

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ABSTRACT

Therapeutic value of the alkylating agent ifosfamide has been limited by major side effects including encephalopathy. Although the underlying biochemical processes of the neurotoxic side effects are still unclear, they could be attributed to metabolites rather than to ifosfamide itself. In the present study, the effects of selected ifosfamide metabolites on indices of neuronal activity have been investigated, in particular for S-carboxymethylcysteine (SCMC) and thiodiglycolic acid (TDGA). Because of structural similarities of SCMC with glutamate, the \( \text{Ca}^{2+} \) response of single mouse cortical neurons to SCMC and TDGA was investigated. SCMC, but not TDGA, evoked a robust increase in \( \text{Ca}^{2+} \) concentration that could be abolished by the \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), but only partly diminished by the N-methyl-D-aspartate receptor antagonist 10,11-dihydro-5-methyl-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801). Cyclothiazide (CYZ), used to prevent AMPA/kainate receptor desensitization, potentiated the response to SCMC. Because activation of AMPA/kainate receptors is known to induce proton influx, the intracellular pH (pHi) response to SCMC was investigated. SCMC caused a concentration-dependent acidification that was amplified by CYZ. Since \( \text{H}^+ \)/monocarboxylate transporter (MCT) activity leads to similar cellular acidification, we tested its potential involvement in the pHi response. Application of the lactate transport inhibitor quercetin diminished the pHi response to SCMC and TDGA by 43 and 51%, respectively, indicating that these compounds may be substrates of MCTs. Taken together, this study indicates that hitherto apparently inert ifosfamide metabolites, in particular SCMC, activate AMPA/kainate receptors and induce cellular acidification. Both processes could provide the biochemical basis of the observed ifosfamide-associated encephalopathy.

Ifosfamide is an oxazophosphorine used in the treatment of cancer in children and adults. Encephalopathy is a serious, sometimes fatal, and limiting side effect of ifosfamide therapy. This adverse reaction is particularly associated with the oral administration of ifosfamide and in the largest series of chemotherapy cycles performed to date (390 cycles in 65 patients), the incidence of encephalopathy was 30% (Cerny et al., 1989). Studies in smaller patient series have reported even higher incidences of encephalopathy up to 100% (Aeblinger et al., 1986). It has been proposed (Visarius et al., 1999) that the metabolism and/or depletion of hepatic glutathione, but this mechanism is still unverified. In recent years, research in this area has focused on the mitochondrial after the systemic

ABBREVIATIONS: SCMC, S-carboxymethylcysteine; \( \text{pH}_i \), intracellular pH; \( \text{Ca}^{2+}_i \), intracellular \( \text{Ca}^{2+} \); BCECF, 2',7'-bis(6-carboxyfluorescein)-5,6-carboxyfluorescein; NMDA, \( N \)-methyl-D-aspartate; AMPA, \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AM, acetoxymethyl ester; SSEC, S-carboxymethylcysteine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; TDGA, thiodiglycolic acid; CYZ, cyclothiazide; MESNA, sodium 2-mercaptoethanesulfonate; MCT, monocarboxylate transporter; MK-801, 10,11-dihydro-5-methyl-5H-dibenzo[a,d]cyclohepten-5,10-imine; \( \alpha \)-CIN, \( \alpha \)-cyano-4-hydroxycinnamate.
investigation of a single case by Küpfer et al. (1994) demonstrated that a glutaric aciduria type II-like mitochondrial biochemical defect was present and that ifosfamide encephalopathy responded successfully to administration of methylene blue, both therapeutically and prophylactically. These same authors (Küpfer et al., 1996) have also proposed a metabolic basis of the encephalopathy that involves flavoprotein inhibition, an intracellular redox shift, and the formation of a complex cyclic amino acid metabolite, thialysine, which they hypothesized as the ultimate neurotoxin. However, this putative metabolite has never been determined in patient samples.

It is known, however, that chloroacetalddehyde may lead to the formation of chloroacetic acid and then to S-carboxymethylcysteine (SCMC), after conjugation with the amino acid cysteine, and that SCMC can account for about 80% of the administered dose of ifosfamide (Küpfer et al., 1996), which is then further degraded metabolically to thiodiglycolic acid (TDGA) (Hofmann et al., 1991). Examination of the SCMC chemical structure (Fig. 1) indicates that it shares a close structural similarity with the excitatory neurotransmitter glutamic acid and might therefore have glutamatergic effects on neurons. In this study, we proposed to examine some pharmacological effects of two ifosfamide metabolites, namely SCMC and TDGA, on single mouse cortical neurons in primary culture.

We show that among the ifosfamide metabolites tested, SCMC selectively activates AMPA/kainate receptors in neurons. In addition, SCMC and the other compounds tested induce substantial cellular acidification that may involve neuronal transport through monocarboxylate transporters. The effect of SCMC on AMPA/kainate receptors and the observed cellular acidification will undoubtedly have important consequences on the central nervous system.

**Experimental Procedures**

**Cell Culture**

Mouse neurons in primary cultures were obtained from brain cortices from 17-day mouse embryos mechanically dissociated in phosphate-buffered saline by successive aspiration through sterile fire-polished Pasteur pipettes. The dissociated cells were centrifuged at 600 rpm for 5 min and then resuspended at a density of 60 to 65,000 cells per cm² in Neurobasal (Invitrogen, Basel, Switzerland) culture medium complemented with 2% B27 solution (Invitrogen), 500 μM glutamine, and 30 μM glutamate according to Brewer et al. (1993). Cells were then plated on glass coverslips coated with poly-L-ornithine (Sigma, Buchs, Switzerland). Cells were used after 12 to 20 days of culture.

**Microscopy**

Experiments were carried out on the stage of an inverted epifluorescence microscope (Carl Zeiss GmbH, Jena, Germany) and observed through a 40× 1.3 numerical aperture oil-immersion Neofluor objective lens (Zeiss). Fluorescence excitation wavelengths were selected using a holographic monochromator (Polychrome II; Till Photonics, Planegg, Germany), and fluorescence was detected using a 12-bit cooled CCD camera (Micromax; Princeton Instruments, Trenton, NJ). Acquisition and digitization of images as well as time series were computer-controlled using the software Metafluo (Universal Imaging, West Chester, PA) running on a Pentium computer (Intel, Santa Clara, CA). The acquisition rate of ratio images was varied between 0.5 and 0.1 Hz. Once loaded with dye, cells were placed in a perfusion chamber designed for rapid exchange of perfusion solutions (Chatton et al., 2000). Up to ~10 individual neurons were simultaneously analyzed in the selected field of view.

**pH Measurements**. pH was measured in single cells on glass coverslips after loading the cells with the pH sensitive fluorescent dye 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF-AM; Teflabs, Austin, TX) as described previously (Chatton et al., 1997). Cell loading was performed at room temperature for 10 min using 1 μM BCECF-AM in a HEPES-buffered balanced solution (see composition below). Fluorescence was sequentially excited at 440 and 480 nm and detected through a 535 nm (35 nm bandwidth) interference filter. Fluorescence excitation ratios (\(F_{480}/F_{440}\)) were computed for each image pixel and produced ratio images of cells that were proportional with pH. In situ calibration was performed after each experiment using a nigericin technique (Thomas, 1984); cells were sequentially perfused with high K⁺ calibration solutions (see composition below) at pH 6.6, 7.1, 7.4, and 7.9 supplemented with 1 to 2 μM nigericin while BCECF ratios were measured. A calibration curve was then generated to convert ratio values into pH values for each individual cell under study.

**Ca²⁺ Measurements**. Ca²⁺ was measured using Fura-2 (Molecular Probes, Eugene, OR) loaded into cells by incubation with 5 μM Fura-2/AM for 30 min at 37°C. Experiments were performed in CO₂/bicarbonate-buffered solutions (see composition below). Calibration of cytosolic signal was accomplished in situ at the end of some experiments as previously described (Kao, 1994). Fluorescence was sequentially excited at 340 and 380 nm and detected at >515 nm.

**Solutions**

CO₂/bicarbonate-buffered experimental solutions contained 135 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl₂, 0.8 mM MgSO₄, 0.78 mM NaH₂PO₄, 25 mM NaHCO₃, and 5 mM glucose bubbled with 5% CO₂/95% air. HEPES-buffered experimental solutions contained 160 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl₂, 0.8 mM MgSO₄, 0.78 mM NaH₂PO₄, 20 mM HEPES, and 5 mM glucose bubbled with air. This saline was supplemented with 1% bovine serum albumin for dye.
loading. pH calibration solutions contained 20 mM NaCl, 120 mM KCl, 10 mM HEPES, 1.3 mM CaCl₂, 0.8 mM MgSO₄, and 0.78 mM NaH₂PO₄ and were adjusted to their respective pH by addition of NaOH.

Materials

Nigericin, TDGA, and sodium 2-mercaptoethanesulfonate were purchased from Fluka (Buchs, Switzerland). S-Carboxylethylcysteine (SCEC) was purchased from Aldrich (Buchs, Switzerland). CNQX, MK-801, and cyclothiazide were obtained from Tocris/ANAWA Trading (Wangen, Switzerland). SCMC and all other substances were obtained from Sigma.

Expression of Data and Statistics

Data are presented as means ± S.E. Student’s t test was performed to assess the statistical significance of results with a p < 0.05 considered as significant. For estimation of EC₅₀ values, nonlinear curve fitting was performed using the Levenberg-Marquardt algorithm implemented in the Kaleidagraph software package (Synergy Software, Reading, PA). The dose-response analysis experiments were fitted using the following equation:

$$R_{\text{obs}} = R_{\text{max}}[S]^n/K + [S]^n + R_{\min}$$

in which $R_{\text{obs}}$ is the observed response; $R_{\text{max}}$ and $R_{\min}$ are maximum and minimum parameters of the response, respectively. [S] is the concentration of transported compound, and $K$ is the agonist concentration that yields half-maximum responses (i.e., EC₅₀), and $n$ is the Hill coefficient.

Results

Ifosfamide Metabolites and Glutamatergic Activity.

Because the chemical structure of SCMC closely resembles that of the excitatory amino acid glutamate (Fig. 1), we tested the possibility that it could also interact with neuronal i onotropic glutamate receptors. Neurons were loaded with the Ca²⁺-sensitive probe Fura-2, and the changes in intracellular Ca²⁺ were monitored after application of the different compounds.

Figure 2 shows typical responses of a single cortical neuron, where SCMC resulted in clear-cut Ca²⁺ response, even though in comparison the response to glutamate (100 μM) was somewhat stronger. SCEC (a structural analog of SCMC), TDGA (the end metabolite of SCMC), and Na⁺ 2-mercaptoethane sulfonate (MESNA), a drug generally co-administered with ifosfamide as a uroprotective agent (Dechant et al., 1991), did not elicit sizable Ca²⁺ responses.

In the next phase, the Ca²⁺ response to SCMC was characterized. Figure 3A shows that the amplitude (nM) of the response to SCMC is concentration-dependent with an apparent EC₅₀ of 1.3 ± 0.1 mM (n = 74 cells from 12 experiments). This concentration dependence appears cooperative with a Hill coefficient of ~2. In this series of experiments, cells that did not respond at low SCMC concentrations generally responded to SCMC at concentrations higher than 1 mM, indicating that some threshold of activation had to be achieved.

The mode of action of SCMC on cortical neurons was investigated by testing the sensitivity of the SCMC-evoked Ca²⁺ response to CNQX and MK-801, classical blockers of AMPA/kainate and NMDA receptors, respectively. Figure 3B depicts an experiment during which the response to SCMC evoked a robust response in a single neuron. Subsequent application of 25 μM CNQX completely abolished the Ca²⁺ response to SCMC. In comparison, application of 10 μM MK-801 displayed only a moderate inhibitory effect. A final SCMC application after washout of the inhibitors shows that the cell remained responsive. This series of experiments indicated that AMPA/kainate receptors rather than NMDA receptors are responsible for the Ca²⁺ response to SCMC. To further verify this conclusion, we used the compound cyclothiazide (CYZ), which prevents the desensitization of AMPA/kainate receptors—but not of NMDA receptors—in the presence of the agonist (Bertolino et al., 1993). Thus, CYZ maintains the channel in an open configuration in the presence of AMPA/kainate receptor agonists and considerably amplifies the response. Figure 3C shows that after a control application of SCMC that resulted in a typical response, 100 μM CYZ (applied first alone) did not induce any detectable response, but when SCMC was coadministered with CYZ, a much larger Ca²⁺ response was observed, further stressing the involvement of AMPA/kainate receptors in the neuronal response to SCMC. Because it had been shown that AMPA/kainate receptor activation was accompanied with cellular acidification in hippocampal neurons and in astrocytes (Rose and Ransom, 1996), pHᵢ was measured during SCMC application. Figure 3D shows that 1 mM SCMC indeed decreased pHᵢ reversibly and that application of 100 μM CYZ massively potentiated this pHᵢ response to SCMC.

Ifosfamide Metabolites and Intracellular Acidification.

Because there is evidence (Hofer, 1995; Foxall et al., 1997) that SCMC interacts with the hepatic lactate transporter MCT2 (see Discussion) and because we observed (data not shown) that the acidification induced by SCMC was reduced but not abolished by CNQX as was the Ca²⁺ response, we investigated the possibility that SCMC and TDGA were substrates of the neuronal monocarboxylate transporters (MCT2).

Monitoring cellular lactate uptake is rendered difficult by the fact that once entered in the cell, lactate is rapidly me-
tabolized and therefore difficult to trace. We chose to follow transport activity through the associated acidification caused by the cotransport of monocarboxylates and protons, as previously described (see e.g., Halestrap and Price, 1999). In a first phase, we validated the technique by measuring lactate transport activity by means of \( pH \) changes in single mouse cortical neurons. Figure 4 shows that both lactate and pyruvate induced rapid dose-dependent and reversible acidifications with an apparent \( K_d \) of 1.3 ± 0.1 mM (9 experiments) and 2.8 ± 0.6 mM (29 cells, 4 experiments), respectively, in agreement with published values (Halestrap and Price, 1999).

SCMC was then tested for its ability to induce intracellular acidification in neurons. Figure 5A shows that SCMC (1 mM) induced intracellular acidification comparable in kinetics and additive to that induced by lactate. The acidification was concentration-dependent with an apparent \( EC_{50} \) of 0.92 ± 0.27 mM (Fig. 5B). In comparison, the compounds SCEC and TDGA, which did not elicit measurable \( Ca^{2+} \) responses, induced acidifications of similar magnitudes (Fig. 6). Like SCMC, SCEC led to an additive acidification with lactate, which may indicate that more than one mechanism is responsible for the \( pH \) response. Among possible mechanisms, there are intracellular targets such as mitochondrial pyruvate transporters (see below). Acidification by TDGA did not appear to be additive with that induced by lactate. For all three compounds, a final application (“recovery”) of lactate at the end of the experiment induced a response comparable with that of the first control application, showing the reversibility of the acidification induced by the compounds.

To examine whether the intracellular acidification evoked by SCMC, SCEC, and TDGA involved \( H^+ \) cotransport of the compounds by MCT, the effect of MCT transport inhibitors was tested. In a first phase, \( \alpha \)-cyano-4-hydroxycinnamate (\( \alpha \)-CIN), a known inhibitor of the MCTs, was used (Halestrap and Price, 1999). However, in our conditions, \( \alpha \)-CIN alone caused a massive acidification in cortical neurons, precluding its use in further experiments. The reason for this acidification is probably the accumulation of cytosolic lactic acid caused by the inhibition of mitochondrial pyruvate transporter—for which \( \alpha \)-CIN is a more potent inhibitor (>100- to 1000-fold). Quercetin, another described inhibitor of MCT, which by itself did not cause significant acidification, was then tested (Volk et al., 1997; Halestrap and Price, 1999). Figure 7 shows that quercetin significantly reduced the response to SCMC by 43%. The response to SCEC was also reduced by ~25% without, however, reaching statistical significance, whereas the response to TDGA was inhibited by ~50%. Taken together, these results indicate that SCMC, SCEC, and TDGA induce substantial cellular acidification that appears to involve more than one mechanism.

**Discussion**

In the present study, we observed that the major metabolite, SCMC, of the alkylating antitumor agent ifosfamide has agonistic effects on AMPA/kainate receptors in mouse cortical neurons, and, like the terminal metabolite TDGA, induces substantial cellular acidification. These effects would undoubtedly interfere with normal central nervous system...
functions and may provide a molecular basis for the observed encephalopathies associated with ifosfamide chemotherapy.

The first observation of this study is that SCMC is an agonist of AMPA/kainate receptors, whereas it only weakly activates NMDA receptors. This element is undoubtedly of high relevance for the understanding of the encephalopathies associated with ifosfamide treatment; as such, SCMC could exert excitotoxic actions (Ambrosio et al., 2000). It should be pointed out that SCMC is routinely prescribed as an oral mucolytic agent, which under normal dosage has not been described to cause neuropathies. It is thus unlikely that SCMC freely crosses the blood-brain barrier. Furthermore, the EC_{50} of \( \frac{1.3 \text{ mM}}{1.0 \text{ mM}} \) Ca^{2+} response to SCMC indicates that relatively high concentrations have to reach the parenchyma to elicit significant effects. Doses of ifosfamide given to cancer patients are frequently 1 to 17.5 g per chemotherapy cycle, and therefore, plasma ifosfamide concentrations reached during chemotherapy might well result in brain interstitial SCMC concentrations in the range where it has agonistic effects on AMPA/kainate receptors. It has been observed in rats (A. Küpper and J. R. Idle, unpublished observations) that administration of high doses of SCMC, unlike those of TDGA, do not produce observable CNS effects, confirming the suspicion that this amino acid derivative does not cross the blood-brain barrier.

Likely sources of the SCMC in the brain are reactions in situ between cysteine and/or glutathione and two-carbon donor molecules such as chloroacetaldehyde and chloroacetic acid. Recently, Saghir et al. (2001) demonstrated that, in male rats administered with \(^{14}\text{C}\)chloroacetic acid, the mean residence time for radioactivity was much higher in the brain than any other tissue measured. It is plausible that chloroacetic acid metabolism in the brain to polar and resident metabolites, presumably SCMC, explain the mean residence times of around 30 h compared with that for the plasma of less than 4 h. At intravenous doses of chloroacetic acid of 60 mg/kg or above, a high proportion of rats entered coma and then rapidly died.

Data on ifosfamide and SCMC have indirectly indicated that SCMC interferes with the hepatic monocarboxylate transporter. Foxall et al. (1997) performed high-resolution proton nuclear magnetic resonance spectroscopy on the urine of 10 nonencephalopathic and 5 encephalopathic patients during their ifosfamide treatment. In the encephalopathic patients, characteristic time-related changes in the excretion profiles of some low molecular weight molecules were observed. Of particular note was a decrease in hippuric acid excretion with a concomitant increase in glycine excretion. Hippuric acid is formed from dietary benzoic acid after MCT2-mediated transport into the liver and subsequent conjugation with glycine. Unfortunately, they did not determine benzoic acid excretion. These observations, together with an enhanced urinary excretion of lactate in the encephalopathic patients, suggest that the encephalopathy is associated with the inhibition of MCTs at the level of the liver. In support of this concept, Hofer (1995) showed that the metabolism of the anticancer drug thiotepa in both adult and pediatric cancer patients proceeded to SCMC and then to TDGA, analogously to ifosfamide. Patients had elevated urinary excretion of benzoic acid, normally only found in trivial concentrations in the urine, with values up to 50 times normal.

Monocarboxylates are transported by MCTs along with protons, the stoichiometry of the cotransport being one monocarboxylate molecule for one proton (Halestrap and Price, 1999). Transport through MCTs is therefore associated with cellular acidification.

In line with the evidence from studies in liver, we found in
the present study that SCMC and TDGA—the latter having no effect on Ca\(^{2+}\)—induced significant cellular acidification that could be partly inhibited by the MCT transport inhibitor quercetin, suggesting the possible involvement of lactate transporters in the acidification. However, the additivity of the pH\(_i\) response to lactate and to the tested substances does not support transport through MCTs as the sole mechanism responsible for the observed acidifications.

Neurons are known to rely mainly on oxidative metabolism to sustain their activity (Magistretti et al., 1999), and glucose has long been thought to be the sole metabolic substrate of neurons in the brain. However, it has been shown also that neuronal activity could be completely preserved in the absence of glucose if neurons were given lactate or pyruvate as metabolic substrates (Schurr et al., 1988). Astrocytes have been shown to release substantial amounts of lactate, originating from the glycolytic processing of glucose (Pellerin and Magistretti, 1994). This set of observations has been encapsulated in an operational model of brain energy metabolism at the cellular level (Magistretti et al., 1999), which suggests the production of lactate by astrocytes in register with synaptic activity and subsequent use of lactate by neurons to fuel the tricarboxylic acid cycle and respiration. Cell-specific expression of MCTs has been found in the membrane of both astrocytes (MCT1) and neurons (MCT2) (Pierre et al., 2000).

As a first functional consequence, access to the metabolic substrate lactate by neurons could be hindered in the presence of SCMC and TDGA, and normal neuronal activity would be inhibited. Indeed, inhibition of lactate transport has been shown to decrease synaptic activity in hippocampal slices (Izumi et al., 1997). In addition, regardless of the mechanism responsible for the acidification, a decreased cellular pH will have the consequence of weakening lactate/proton cotransport. These metabolites once taken up in cells may also be cytotoxic [e.g., by interfering with mitochondrial function (Marthaler et al., 1999) or with mitochondrial pyruvate transporter]. Thus, SCMC and TDGA may interfere with lactate uptake and energy metabolism in neurons through different mechanisms.

It is possible that SCMC may exist at background levels in various body compartments due to human exposures to envi-

**Fig. 5.** Cellular acidification induced by SCMC. Panel A, experimental trace comparing the acidification induced by 5 mM lactate with that induced by 1 mM SCMC alone or the combination of lactate plus SCMC. Panel B, concentration dependence of the SCMC-induced acidification yielding an apparent EC\(_{50}\) of 0.92 ± 0.27 mM (n = 51 cells from seven experiments).

**Fig. 6.** Comparison of SCMC-, SCEC-, and TDGA-induced acidification with the lactate-evoked acidification. The acidification (ΔpH\(_i\)) induced by 1 mM SCMC (panel A), 1 mM SCEC (panel B), and 1 mM TDGA (panel C) alone or in combination with 5 mM lactate is compared with that induced by 5 mM lactate alone. Data are means ± S.E.M. The number of cells and experiments are indicated in the graph.
mental toxicants. Analysis of normal urine from persons with no history of exposure to noxious chemicals revealed that 39 of 40 volunteers excreted 0.2 to 2.0 mg/day TDGA in their urine (Vågbo et al., 1998). Likewise, Müller et al. (1979) described excretion values of TDGA in the same range. It is not known if "endogenous" TDGA derived from SCMC. Nevertheless, a second potential source of TDGA is the two cyclic sulfur-containing dicarboxylic acids, hexahydro-1,4-thiazepine-3,5-dicarboxylic acid (cyclothionine) and thiomorpholine-3,5-dicarboxylic acid, which are also present in normal human urine (Matarese et al., 1987). Ring opening of these acids followed by decarboxylation might be expected to yield TDGA as has been demonstrated for thiomorpholine in Mycobacterium aurum cultures (Combourieu et al., 1998). By what other means might the human brain be exposed to this potential neurotoxin SCMC? Electrophilic two-carbon donors can react with cysteine to yield S-substituted cysteines, which will ultimately be oxidized to SCMC. For example, the industrial chemicals and solvents 2,2'-bis-(chloroethyl)ether (Lingg et al., 1982), vinyl chloride (Chen et al., 1983), 1,2-dichloroethane (ethylene dichloride; Cheever et al., 1990), and acrylonitrile (Fennell et al., 1991) are all metabolized to SCMC and TDGA. Interestingly, the toxicity profiles of all of these compounds comprise a significant component of neurotoxicity (e.g., vegetative dysfunction; Liubchenko et al., 1997), and this compound has been evaluated by a working group to be a possible human neurotoxin (Simon- sen et al., 1994). All of these low molecular weight compounds

![Fig. 7](image-url) Inhibition of the acidification induced by SCMC, SCEC, and TDGA by quercetin. The figure depicts the effects of 50 μM quercetin on the acidification induced by 5 mM lactate (panel A), 1 mM SCMC (panel B), 1 mM SCEC (panel C), and TDGA (panel D). Data are means ± S.E.M., *** p < 0.001 using the paired t test (N.S.). The number of cells and experiments are indicated in the graph.

![Fig. 8](image-url) Biotransformation of SCMC to TDGA via oxidation or transamination pathways. The figure shows the common role of lactate dehydrogenase in the interconversions of 3-(S-carboxymethylthio)lactic acid with 3-(S-carboxymethylthio)pyruvic acid with 3-(S-carboxymethylthio)pyruvic acid, lactic acid with pyruvic acid, and the sensitivity of both these interconversions to the cellular NAD+/NADH ratio.

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may act by readily accessing the brain and generating in situ the resident metabolites SCMC and/or TDGA.

Finally, it should be stated that a scheme could be envisaged whereby SCMC and TDGA are able to interact with lactate and pyruvate in the brain. Figure 8 shows the metabolic biotransformations necessary to convert SCMC to TDGA. The intermediates 3-(S-carboxymethyl)lactuic acid and 3-(S-carboxymethylthio)pyruvic acid are formed from SCMC by the processes of oxidative deamination and transamination, respectively, and 3-(S-carboxymethyl)lactuic acid is a principal metabolic intermediate of SCMC (Meese and Fischer, 1990; Hofmann et al., 1991). An overload of chloroacetaldehyde and its conversion by aldehyde dehydrogenase to chloroacetic acid will generate an excess of NADH at the expense of NAD⁺, as discussed by Küpper et al. (1996), and may promote the formation not only of lactate from pyruvate but also of 3-(S-carboxymethyl)lactuic acid from 3-(S-carboxymethylthio)pyruvic acid. The consequence of this may be to hinder further the uptake of the essential neuronal fuel, lactate, by MCTs.

The understanding of the potential neurochemical mechanisms of ifosfamide encephalopathy, in particular the identification of discrete receptor and transporter types with which the major ifosfamide metabolites SCMC and TDGA interact, opens new horizons of investigation for the pharmacological manipulation of this adverse drug reaction, both therapeutic and prophylactic.

In conclusion, this study shows that certain ifosfamide metabolites, in particular SCMC, cause activation of AMPA/kainate receptors and induce cellular acidification, which may constitute the mechanisms responsible for the encephalopathy that is frequently associated with ifosfamide treatment. This may represent a novel pathway of neurotoxicity for a number of different compounds that are able to generate these same metabolic products in vivo.

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References


